STANDARD OPERATING PROCEDURE FOR ANALYZING ALCOHOL ETHOXYLATES AND ALKYLPHENOL ETHOXYLATES USING LC-MS

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1.0 Disclaimer

This standard operating procedure has been prepared for use of the Environmental Sciences Division, Environmental Chemistry Branch, NERL, ORD of the U.S. Environmental Protection Agency and may not be specifically applicable to the activities of other organizations. **THIS IS NOT AN OFFICIAL EPA APPROVED METHOD.** This document has not been through the Agency's peer review process or ORD clearance process.

2.0 Purpose (Scope and Application)

This document describes the procedure for the determination of alcohol ethoxylates (AEOs), nonylphenol ethoxylates (NPEOs), and octylphenol ethoxylates (OPEOs) in water samples by solid-phase extraction (SPE) and liquid chromatography-mass spectrometry (LC-MS).

3.0 Method Summary

3.1 The method employs high-performance liquid chromatography (HPLC) coupled with positive electrospray ionization (ESI+) mass spectrometry (MS) for the determination of AEOs, NPEOs, and OPEOs in aqueous matrices.

3.2 Aqueous samples are first flowed through Oasis HLB SPE cartridges (polystyrene-divinylbenzene- N-vinylpyrrolidone terpolymer resin) to extract the ethoxylated compounds from solution before concentrating the samples to 0.5 mL.

3.3 Target compounds are identified by retention time and m/z in the full scan operating mode. Compounds are quantified using an external standard calibration.

4.0 Interferences

4.1 Ethoxylated compounds are common primary ingredients used in many detergents. All glassware must be washed with detergents free from ethoxylated alcohols. Powdered Alconox does not contain ethoxylated alcohols, but any comparable detergent free from these interferences may also be used.

4.2 Method interferences can be caused by contaminants in glassware, solvents, and other apparatus producing discrete artifacts or elevated baselines. These materials are routinely demonstrated to be free from interferences by analyzing laboratory reagent blanks and method blanks under the same conditions as the samples.

4.3 Matrix interferences may be caused by contaminants that are co-extracted from the sample.

5.0 Safety

5.1 All of the chemicals used in this procedure should be handled only while using proper personal protective equipment such as gloves, lab coats, safety glasses and fume hoods. The analyst should review the Material Safety Data Sheet for each chemical in this procedure so that safe working conditions can be achieved. AEOs, NPEOs, and OPEOs are skin irritants.

5.2 The toxicity of each reagent used in this method may not have been fully established. Each chemical should be regarded as a potential health hazard, and exposure should be kept as low as reasonably achievable.

5.3 Waste must be disposed of in appropriate waste containers. Contact the onsite SHEM Program Manager to dispose of full waste containers.

5.4 Exhaust fumes from the HPLC-MS must be properly vented.

5.5 All applicable safety and compliance guidelines set forth by the EPA and by federal, state, and local regulations must be followed during the performance of this SOP. Stop all work in the event of a known or potential compromise to the health and safety of any person and immediately notify the SHEM Program Manager and other appropriate personnel.

5.6 Analysts must be cognizant of all instrumental hazards (i.e., dangers from electrical shock, heat, or explosion).

6.0 Reagents/Chemicals/Gases

6.1 HPLC-grade Methanol

6.2 HPLC-grade Water

6.3 Deionized (DI) water: in-house 18 MΩ-cm DI water

6.4 Methyl t-butyl ether (MTBE)

6.5 AEO standards. Currently, only technical mixtures of ethoxylated compounds are available, most likely due to the production conditions of ethoxylates from linear alcohols through reaction with ethylene oxide (EO). Neodol 25-9 (Shell) is a commercial formulation that is composed of C_{12}-C_{15} homologues, with an average ethoxylation of 9 units (ethoxymers range from approximately 0-23 EO units).

Based on MS intensities, the composition consists of approximately 20% C12, 30% C13, 25% C14, and 25% C15 EOs.

6.6 NPEO standards. Tergitol NP-9 is a commercial formulation that contains an average ethoxylation of 9 units.

6.7 OPEO standards. Triton X-100 is a commercial laboratory detergent that consists of a distribution of OPEO ethoxymers.

6.8 AEO pure standards from Sigma for use as surrogates: Sigma provides a small number of certain AEOs in pure form, including C6EO5, C8EO4, C8EO5, C10EO4, C10EO6, C12EO3, and C12EO4. C12EO4 was used as the surrogate in this method because its properties were closest to the C12-C15 EOs in the Neodol 25-9. Standards dissolved in methanol.

6.9 Isopropyl alcohol

6.10 Ammonium acetate

6.11 Leucine-enkephalin std, for tuning the MS

6.12 Sodium formate, for mass calibrating the MS

7.0 Equipment and Supplies

7.1 HPLC-MS system: (Waters LCT Premier)

7.2 HPLC column (Acquity UPLC BEH C18 1.7 µm, 2.1 x 100 mm). Other columns may be used if they provide sufficient retention and separation of the target analytes.

7.3 Variable volume standard pipettors (0.5 -10 µL, 20-200 µL, 100-1000 µL)

7.4 Pipet tips

7.5 Glass beakers, volumetric flasks, sized as appropriate

7.6 Disposable borosilicate Pasteur pipets

7.7 Ultra-high-purity grade compressed nitrogen

7.8 1 mL autosampler vials with PTFE/silicone septa

7.9 Disposable 0.45 µm syringe tip filters, if needed to remove suspended solids

7.10 Filtering apparatus for filtering large volume samples using glass fiber filter discs (type 934-AH, or equivalent), if necessary

7.11 TurboVap Concetrator, for concentrating samples

7.12 Autotrace SPE Workstation

7.13 Oasis HLB SPE cartridges (200 mg, 6 cc size)

8.0 Sample Collection, Preservation, and Storage

8.1 This SOP does not describe sample collection procedures; however, the following guidelines are followed once samples are received in the laboratory.

8.2 Samples must be stored at 4°C in a designated sample refrigerator.

8.3 Holding time studies have not been performed on these analytes; however, samples should be analyzed as soon as possible, and within 28 days.

9.0 Quality Control

9.1 The following are relevant QC criteria for this method.

Table 1. Data Quality Indicators of Measurement Data.

QC Check	Frequency	Completeness	Precision	Accuracy	Corrective Action
Initial 5-point calibration	Prior to sample analysis	100%	RSD≤20%	$R^2 > 0.99$	No samples will be run until calibration passes criteria.
Laboratory blank	One per batch of samples[a]	100%	N/A	< PQL[b]	Inspect the system and reanalyze the blank. Samples must be bracketed by acceptable QC or they will be invalidated.
Instrument blank	One at beginning of each 8-hr analytical day, one at beginning of each batch of samples[a], and one at end of analytical day	100%	N/A	< PQL[b]	Inspect the system and reanalyze the blank. Samples must be bracketed by acceptable QC or they will be invalidated.
Laboratory control sample (LCS)	One per batch of samples[a]	100%	RPD≤30%[c]	± 30% of known value	Check the system and reanalyze the standard. Re-prepare the standard if necessary. Recalibrate the instrument if the criteria cannot be met. Samples must be bracketed by acceptable QC or they will be invalidated.

Laboratory fortified matrix	One per batch of samples[a]	100%	RPD≤30%[c]	>60% recovery	Review data to determine whether matrix interference is present. If so, narrate interference and flag recovery. If no interference is evident, verify the instrument is functioning properly by running a lab blank. Reanalyze recollected sample to verify recovery. Samples must be bracketed by acceptable QC or they will be invalidated.
Laboratory replicates	One per batch of samples[a]	100%	RPD≤30%[c]	>60% recovery	Inspect the system, narrate discrepancy. Samples must be bracketed by acceptable QC or they will be invalidated.
Continuing calibration verification (CCV)	One at beginning of each 8-hr analytical day, one at beginning of each batch of samples[a], and one at end of analytical day	100%	RSD≤30%[c]	± 30% of known value	Inspect system and perform maintenance as needed. If system still fails CCV, perform a new 5-point calibration curve. Samples must be bracketed by acceptable QC or they will be invalidated.
Laboratory fortified blank	One per batch of samples[a]	100%	N/A	>60% recovery	Inspect the system and reanalyze the standard. Re-prepare the standard if necessary. Re-calibrate the instrument if the criteria cannot be met. Samples must be bracketed by acceptable QC or they will be invalidated.
Minimum detection limit	Each chemical	100%	TBD for each HF chemical	TBD for each HF chemical	TBD for each HF chemical

[a]Batch of samples not to exceed 20
[b]PQL=practical quantitation limit, 5 times the MDL
[c]Precision among replicates if more that 1 batch of samples are analyzed. RSD may be applicable if more than 2 replicates are analyzed.

10.0 Calibration and Standardization

10.1 Tune the MS according to manufacturer's directions using Leucine-enkephalin.

10.2 Mass calibrate the MS according to manufacturer's directions using sodium formate solution.

10.3 Tuning to determine the correct system settings (e.g., cone voltage, desolvation temperature, source temperature, etc.) for particular analytes is done as needed and according to the manufacturer's directions. Representative settings for the analytes in this method are listed in section 11.4.

10.4 Record all instrument maintenance in the instrument maintenance log book.

10.5 Suggested concentrations for the initial calibration levels are 25 to 1000 ppb NPEO, OPEO, and C12EO4, and 100 to 4000 ppb Neodol 25-9 (consists of C12-C15 ethoxylates). A minimum of 5 calibration levels must be used.

10.6 Linear calibration may be used if the coefficient of determination, r^2, is >0.99 for the analytes and all continuing calibrations and calibration verifications pass.

10.7 Quadratic calibration may be used if the linear fit fails and the quadratic coefficient of determination, r^2, is >0.99 for the analytes. Each calibration point used to generate the curve must have calculated percent deviation less than 25% from the generated curve.

11.0 Procedure

11.1 Glassware cleaning

11.1.1 Prepare soapy bath with hot water and approximately 1 tsp Alconox detergent. Scrub glassware with bottle brushes and/or pipe cleaners until visibly clean (do not scratch glassware with metal from brushes). Rinse glassware first with non-DI water, and then with DI water. Soak glassware in acid bath (3 mL HCl, 3 mL HNO_3, 4 L water, pH 1-2) overnight. Remove glassware and rinse with Ultrapure DI water. Rinse glassware with methanol and air dry. Place glassware in oven at 100°C for 6 hours.

11.2 Sample preparation

11.2.1 Add an appropriate amount of surrogate to a known volume of aqueous sample for extraction. For the purposes of this method, the addition of 100 ng of C12EO4 to 500 mL sample worked well.

11.2.2 If aqueous sample contains suspended solids, first filter using filtering apparatus and glass fiber discs. Ensure that the glass fiber disc is rinsed well with water, followed by MTBE.

11.3 Solid-phase extraction

11.3.1 Precondition the Oasis HLB cartridges by loading 20 mL isopropyl alcohol through each cartridge. Discard isopropyl alcohol.

11.3.2 Load cartridges into Autotrace SPE Workstation, and condition the cartridges with 5 mL methanol, followed by 5 mL water, both at a flow rate of 5 mL/min.

11.3.3 Load 500 mL aqueous sample through the SPE cartridges at a flow rate of

5 mL/min. Rinse the volumetric flasks with 50 mL water, and load this water through the SPE cartridges as well.

11.3.4 Rinse the cartridges with 2 mL water at a flow rate of 3 mL/min, and dry the cartridges with N_2 for 30 min.

11.3.5 Elute with 5 mL 2:2:1 methanol/acetone/ethyl acetate and then with 5 mL 90:10 MTBE/methanol, both at a flow rate of 1 mL/min.

11.3.6 Quantitatively transfer the eluate from the Autotrace collection tube to a TurboVap tube. Concentrate and solvent exchange the eluate into methanol using the TurboVap Concentrator. Concentrate to 0.5 mL methanol.

11.3.7 Transfer the concentrated sample with Pasteur pipet to an autosampler vial.

11.3.8 Filter the samples, if necessary, with a syringe filter prior to MS analysis.

11.4 LC-MS analysis

11.4.1 Mobile phase A consists of 2 mM ammonium acetate in HPLC grade water. Mobile phase B consists of 2 mM ammonium acetate in HPLC grade acetonitrile.

11.4.2 The following LC gradient is used to analyze ethoxylated alcohols (column temperature of 30°C):

Time (min)	Flow rate (mL/min)	%A	%B
Initial	0.25	95	5
1	0.25	95	5
5	0.25	60	40
11	0.25	25	75
12	0.25	5	95
14	0.25	5	95
15	0.25	95	5
16	0.25	95	5

11.4.3 MS analysis conditions: 0.2 s scans from 0-16 min, from 40 – 1500 m/z in ESI+ "W" mode. Capillary voltage set to 3200 V, sample cone set to 55 V. Desolvation temperature of 250°C and source temperature of 100°C. Cone gas flow rate at 30 L/h, and desolvation gas flow rate at 500 L/h.

11.4.4 Load samples into the Acquity autosampler. In the MassLynx software, click "File->New", and a new sample queue will be visible. Enter an

appropriate file name, file text (sample ID), vial location in autosampler, injection volume (10 µL for each sample), and the appropriate MS and inlet files (containing the above MS parameters and LC conditions, respectively) for each sample.

11.4.5 Under the "Instrument" tab in MassLynx, click on "Inlet Method". In the window that pops up, click on the "Acquity Additional Status" tab. Launch the Acquity UPLC Console by clicking on the circular icon on the right-hand side. Prime the LC solvents by clicking "Control-> Start up system". Ensure that all of the solvents, seal wash, strong wash, weak wash, and sample syringe are primed for at least 5 cycles and click "Start" to prime.

11.4.6 Ensure that the MS has been warmed up with the appropriate method (i.e., capillary and sample cone voltages, temperatures, etc.) for at least one hour before sample injection. Ensure that the gas flow to the MS is on.

11.4.7 In MassLynx, click the "play" button at the top of the screen to start the sample queue.

11.4.8 In MassLynx, under "Edit Shutdown or Startup", ensure that the box next to "Enable shutdown after batch" is checked, with the following method loaded: C:\MassLynx\ShutDown\ShutDownESI_ACE.acl". This ensures that the MS goes into standby mode, that the gases turn off, and that the LC pumps also turn off after the batch of samples has successfully run.

11.5 Data Analysis

11.5.1 Use QuanLynx within the MassLynx software to pick and integrate peaks for each of the analytes. Refer to the manufacturer's instructions for how to create a QuanLynx method for integration. Note that at the time of this writing, individual ethoxymer compositions of the Neodol 25-9, NPEO technical mixture, and OPEO technical mixtures were not known. Therefore, the full scan chromatographic peaks that result from the summed distribution of ethoxymers that provide the highest intensity from the calibration standards of each of the technical mixtures are used for quantitation purposes. The masses of each of the following ethoxymers were input into the QuanLynx method for quantitation purposes. Note that QuanLynx can only accept 10 m/z values per analyte.

Class of ethoxylate	Range of ethoxymers used for quantitation
C12	EO7-EO16
C13	EO7-EO16
C14	EO7-EO16

C15	EO6-EO15
NP	EO8-EO17

11.5.2 After running the QuanLynx method, inspect each chromatographic peak to ensure that the peak has been integrated properly. Because the peaks may be jagged due to 10 analytes per peak, it is usually necessary to correct the peak integration manually.

11.5.3 Identify and confirm the presence of target analytes in the samples by matching the expected m/z values and retention times of the target analytes. The retention time window of the analytes must be within 10% of the retention time of the analyte in the midpoint calibration standard.

11.5.4 Quantitate the amounts of each analyte using the external standard calibration curve.

11.5.5 Calculate the extraction recovery based on the recovery of the surrogate:

$$\%R = 100\% \times \frac{C_m}{C_{srm}}$$

Where:
%R = percent recovery of surrogate
C_m= measured concentration of standard reference material
C_{srm} = actual concentration of standard reference material

Note: During calculations, take into account the concentration factor from the 500 mL sample down to 0.5 mL following extraction/concentration.

11.5.6 Correct the calculated values of the target analytes with the extraction recovery of the surrogate:

$$C_c = C_m/(\%R/100)$$

Where:
C_c = surrogate-corrected concentration
C_m= measured concentration of analyte
%R= percent recovery of surrogate

11.5.7 Calculate the spike recoveries:

$$\%R = 100\% \times \frac{(S - U)}{C_{sa}}$$

Where:
%R = percent recovery
S = measured concentration in spiked aliquot
U = measured concentration in unspiked aliquot
C_{sa} = actual concentration of spike added

12.0 Method Performance

12.1 Method performance is evaluated based on the criteria in Table 1.

12.2 MDLs have not been determined for these compounds yet. Reporting limits are 0.05 µg/L for C12EO7-16, C13EO7-16, C14EO7-16, C15EO6-15, and NPEO8-17.